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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF: ROBERT E. JOHNSTON, ET AL. ART UNIT:
SERIAL NO.: 10/683,781 EXAMINER
:

FILING DATE: OCTOBER 10, 2003
FOR: ALPHAVIRUS RNA REPLICON SYSTEMS

DECLARATION GEORGE V. LUDWIG, PH.D., 37 C.F.R. §1.608(b)

ASSISTANT COMMISSIONER FOR PATENTS
PO BOX 1450
ALEXANDRIA, VA 22313-1450

SIR:

I, George V. Ludwig, Ph.D., do hereby declare and state:

1. I am the Interim Science Director at the United States Army Medical Research Institute of Infectious Diseases (USAMRIID). I have a Ph.D. in Veterinary Science and Entomology from the University of Wisconsin at Madison and have over 20 years experience in rodent studies with viral agents, including Venezuelan Equine Encephalitis (VEE) virus and related alphaviruses.

2. In the time period from November 30, 1994 through May 23, 1995 my position was Chief of the Department of Cell Culture and Hybridoma Production at USAMRIID, where I contributed, through my investigative work, to developing the Alphavirus RNA Replicon Systems of U.S. Patent Application No. 10/683,781 ("the '781 application"), and specifically, to the subject matter of Claim 116, which I understand reads as follows: A method of producing an immune response in a subject, comprising:

administering to the subject an effective amount of a composition comprising a population of infectious, defective alphavirus particles in a pharmaceutically acceptable carrier, wherein each particle comprises one or more heterologous RNA sequence(s) encoding an immunogen; and further wherein the population contains no detectable replication-competent alphavirus particles as determined by passage on permissive cells in culture.

3. The following is a chronological listing of my laboratory activities related to developing the Alphavirus RNA Replicon Systems in the time period from November 30, 1994 through May 23, 1995. In preparing this declaration I reviewed the following documents:

Exhibit O	Notes of Experiment Designator L9412221, Initial Lassa Replicon Study December 22, 1994 through July 11, 1995
Exhibit P	Approved Protocol V96-01, Vaccine Immunogenicity and Efficacy Testing
Exhibit Q	Notes of Experiment Designator L9506291, Experiment two of Lassa Replicon in mice and initial HA replicon study in mice, June 29, 1995 - June 16, 1996.

4. Prior to and during the aforementioned time period, I conducted research and experimentation as a scientist in the Virology Division of USAMRIID at Fort Detrick in Frederick, Maryland. My activity during this time period is recorded in my own laboratory notes which I generated on my computer during the course of my investigations. I generated entries directly in my laboratory notes such that I can attest to their substantive accuracy.

5. These notes provide a detailed history of laboratory activity associated with Experiment L9412221, entitled "Initial Studies of Lassa Replicon (Lassa Nucleocapsid) in Mice" (Exhibit O). This animal study was ongoing and continuous in nature, each day involving one or more separate activities for the study. Daily observation of the animals is a routine part of animal husbandry and a requirement of any animal study in the Virology Division of USAMRIID (see, e.g., Exhibit P, general pp. 5-11, and D, 1 on page 11).

6. In summary, the study involved generating an immune response to the Lassa fever virus nucleoprotein (LSN) by immunizing mice with replication defective VEE-LSN replicon

particles. Mice were immunized with the particles in a pharmaceutically acceptable carrier on the first date of the study (Exhibit O, page 1). Thus, the method that is the subject matter claimed in Claim 116 was performed on December 22, 1994. A second animal study with two different alphavirus replicons was initiated on June 29, 1995, experiment designator L9506291 (Exhibit Q; Lassa replicon and HA replicon). A detailed listing of the daily activity involved in this study is described below.

7. Mice for these experiments were common commercial strains and inoculation was performed intraperitoneally and subcutaneously. All animals used in the protocol were bled from the retroorbital sinus. All animals were housed and cared for according to the NIH "guide" or the Veterinary Medicine Division Standard Operating Procedure. Following these guidelines, animals were observed daily for general health to ensure humane treatment and satisfactory general conditions (Exhibit P, page 11), which was done by me or colleagues/technicians working directly under my supervision. Animals were euthanized at the first sign of a terminal illness or major distress. Exhibit P is a copy of the large scale study protocol that was conducted subsequent to the experimental studies described below. The protocols set forth in Exhibit P are consistent with the protocols used in experimental small scale studies of the Lassa replicon.

8. The experimental work reflected in my computer generated notes occurred predominantly in the V3 portion of the V3V4 Virology Laboratory at Fort Detrick, in Room V307 of Building 1425. The V3V4 laboratory is a self-contained and safe environment for conducting virology experiments, which is a biosafety requirement for experiments involving VEE.

9. Prior to November 30, 1994 I had conversations with Peter Pushko and Jonathan Smith (Pushko and Smith, respectively, herein) about the replicon system with the understanding that the successful construction of infectious replication defective VEE-LSN particles would need

to be tested in vivo to provide in vivo confirmation of the ability of the RNA encoding the immunogen to direct expression of the LSN antigen sufficient to produce an immune response, which had been previously established with recombinant VEE-baculovirus constructs. I began designing the first VEE-LSN animal study (experimental designator L9412221, Exhibit O) approximately 6-8 weeks prior to the first immunization on December 22, 1994.

10. After the first replicon study was initiated but prior to May 23, 1995, Pushko and Smith informed me that additional infectious replication defective particles had been produced and requested they be studied in vivo. Thus, I began designing the second replicon animal study, experimental designator L9506291, which involved two different VEE-derived replicon particles: (1) LSN replicon particles produced with helper sequences designated P21-1 and P24-3; and (2) a replicon expressing the HA antigen of influenza A virus. The mice in this study were first immunized on June 29, 1995.

11. In my opinion, the method described in Claim 116 of the '781 application had been performed no later than January 16, 1995, which was the date I first documented by ELISA a positive anti-LSN immune response in mice that were immunized December 22, 1994.

12. **DETAILED ACTIVITY RECORD:**

November 30, 1994 through December 22, 1994: I was involved in the preparations for the first experimental animal study with the Lassa Replicon. This would have included the routine steps necessary to initiate a small scale study, including species selection and having the animals in the V3V4 lab prior to commencing the study.

December 22, 1994 (Thursday): I commenced the experimental study of the Lassa replicon, experimental designator L9412221 (Exhibit O), and animal protocol V94-03 was adopted. The replicon particles in this study were generated using the Lassa replicon (or LSNREP) and a replicon helper designated P5-1. I obtained the replicon titer for this study from Pushko. The inoculation protocol for the study was as follows. I made dilutions from the titer and divided mice into groups of 5. Each mouse was inoculated with 0.5 ml of LSN replicon particles in a pharmaceutically acceptable carrier. The inoculum was divided and injected intraperitoneally and subcutaneously. (Exhibit O, ¶1).

December 23, 1994 (Friday): I maintained animal husbandry in the Lassa Replicon study for the next step that occurs on December 29, 1994.

December 24, 1994 (Saturday): I maintained animal husbandry in the Lassa Replicon study for the next step that occurs on December 29, 1994.

December 25, 1994 (Sunday - Christmas): I maintained animal husbandry in the Lassa Replicon study for the next step that occurs on December 29, 1994.

December 26, 1994 (Monday): I maintained animal husbandry in the Lassa Replicon study for the next step that occurs on December 29, 1994.

December 27, 1994 (Tuesday): I maintained animal husbandry in the Lassa Replicon study for the next step that occurs on December 29, 1994.

December 28, 1994 (Wednesday): I maintained animal husbandry in the Lassa Replicon study for the next step that occurs on December 29, 1994.

December 29, 1994 (Thursday): I performed a 7-day post-inoculation bleed of the mice. Serum was taken and samples were stored at -20°C in the freezer of Room 304. (Exhibit O, ¶2).

December 30, 1994 (Friday): I maintained animal husbandry in the Lassa Replicon study for the next step that occurs on January 5, 1995.

December 31, 1994 (Saturday): I maintained animal husbandry in the Lassa Replicon study for the next step that occurs on January 5, 1995.

January 1, 1995 (Sunday - New Years Day): I maintained animal husbandry in the Lassa Replicon study for the next step that occurs on January 5, 1995.

January 2, 1995 (Monday): I maintained animal husbandry in the Lassa Replicon study for the next step that occurs on January 5, 1995.

January 3, 1995 (Tuesday):I maintained animal husbandry in the Lassa Replicon study for the next step that occurs on January 5, 1995.

January 4, 1995 (Wednesday): I maintained animal husbandry in the Lassa Replicon study for the next step that occurs on January 5, 1995.

January 5, 1995 (Thursday): I performed a 14-day post-inoculation bleeding. Serum samples were kept at -20°C in a freezer in Room 304. (Exhibit O, ¶3).

January 6, 1995 (Friday): Maintained the ongoing Animal Study of the Lassa replicon by performing animal husbandry before the next step in this study occurring on January 12, 1995.

January 7, 1995 (Saturday): Maintained ongoing Animal Study of Lassa replicon with animal husbandry for the next step occurring on January 12, 1995.

January 8, 1995 (Sunday): Maintained ongoing Animal Study of Lassa replicon with animal husbandry for the next step occurring on January 12, 1995.

January 9, 1995 (Monday): Maintained ongoing Animal Study of Lassa replicon with animal husbandry for the next step occurring on January 12, 1995.

January 10, 1995 (Tuesday): Maintained ongoing Animal Study of Lassa replicon with animal husbandry for the next step occurring on January 12, 1995.

January 11, 1995 (Wednesday): Maintained ongoing Animal Study of Lassa replicon with animal husbandry for the next step occurring on January 12, 1995.

January 12, 1995 (Thursday): I performed the 21-day post-inoculation bleed. Samples were kept at -20°C in freezer in Room 304. (Exhibit O, ¶4).

January 13, 1995 (Friday): Performed immunofluorescence assay (IFA) on Lassa spot slides. The assay was completed by Peter Pushko. (Exhibit O, ¶5).

January 14, 1995 (Saturday): Maintained ongoing Animal Study of Lassa replicon with animal husbandry for the next step occurring on January 15, 1995.

January 15, 1995 (Sunday): Started ELISA plates for VEE and Lassa serology. Antigens prepared included Lassa-R997 Lassa (Josiah) used at 1:1000. VEE antigen was VEE/L8. The diluent included carbonate and bicarbonate buffer. Three ELISA plates for each of the Lassa and VEE antigens were prepared. The plates were wrapped in cellophane and incubated overnight at 4°C. (Exhibit O, ¶6).

January 16, 1995 (Monday - Martin Luther King Holiday): Completed the ELISA for Lassa/VEE. On this day I documented the first detection of an anti-Lassa immune response *in vivo*. Protocol was ELISA 2.PRO. Serum dilutions were prepared at 1:50, 1:150, 1:450, 1:1350, 1:4050, 1:12150, 1:36450, and 1:109350. (Exhibit O, ¶7).

January 17, 1995 (Tuesday): Maintained ongoing Animal Study of Lassa replicon with animal husbandry for the next step occurring on January 18, 1995.

January 18, 1995 (Wednesday): I conducted the 28-day post-inoculation bleed, which was prior to giving booster. Samples were stored at -20°C in a freezer in Room 304. Also administered the 28-day post-inoculation booster shot. The replicon for the booster was the Lassa replicon produced with helper P5-1. Animal protocol V94-03 was followed. The replicon titer was from Peter Pushko and mice were inoculated with either undiluted samples or a 10^{-2} dilution. Five mice were in each group with 0.5 ml inoculate given per mouse. In the dilution group, one mouse was killed after attempting to bite Peter Pushko. (Exhibit O, ¶8).

January 19, 1995 (Thursday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on January 24, 1995.

January 20, 1995 (Friday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on January 24, 1995.

January 21, 1995 (Saturday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on January 24, 1995.

January 22, 1995 (Sunday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on January 24, 1995.

January 23, 1995 (Monday): Maintain ongoing animal study of Lassa replicon with animal husbandry to the next step on January 24, 1995.

January 24, 1995 (Tuesday): Started ELISA plates for VEE and Lassa serology. Antigens used include the Lassa R997 and VEE L8. Normal Vero cell lysate used at 1:1000. Diluent used was carbonate/bicarbonate buffer. Prepared four ELISA plates for each of all the antigens. The plates were wrapped in cellophane and incubated overnight at 4°C. (Exhibit O, ¶9).

January 25, 1995 (Wednesday): Completed ELISA of the Lassa and VEE antigens. The protocol used was ELISA2.PRO. Serum dilutions were prepared at 1:100, 1:200, 1:400, 1:800, 1:1600, 1:3200, 1:6400, 1:12800. (Exhibit O, ¶10).

January 26, 1995 (Thursday): Performed 35 day post-inoculation bleed. Samples were stored at -25°C in a freezer in Room 304. (Exhibit O, ¶11).

January 27, 1995 (Friday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on February 2, 1995.

January 28, 1995 (Saturday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on February 2, 1995.

January 29, 1995 (Sunday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on February 2, 1995.

January 30, 1995 (Monday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on February 2, 1995.

January 31, 1995 (Tuesday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on February 2, 1995.

February 1, 1995 (Wednesday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on February 2, 1995.

February 2, 1995 (Thursday): Performed 42-day post-inoculation bleed. Samples were stored at -20°C in a freezer in Room 304. (Exhibit O, ¶12).

February 3, 1995 (Friday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on February 9, 1995.

February 4, 1994 (Saturday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on February 9, 1995.

February 5, 1995 (Sunday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on February 9, 1995.

February 6, 1995 (Monday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on February 9, 1995.

February 7, 1995 (Tuesday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on February 9, 1995.

February 8, 1995 (Wednesday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on February 9, 1995.

February 9, 1995 (Thursday): Performed 49-day post-inoculation bleed. Samples stored at -20°C in a freezer in Room 304. ELISA plates were started for VEE and Lassa serology. Antigens included the Lassa R997 and the VEE L8 of December 10, 1993. Normal Vero cell lysate used. Diluent was carbonate/bicarbonate buffer. Two ELISA plates for each of all the antigens was prepared. The plates were wrapped in cellophane and incubated overnight at 4°C. (Exhibit O, ¶13).

February 10, 1995 (Friday): The ELISA was completed as per protocol in ELISA2.PRO. This

ELISA demonstrated for the second time the generation of a positive immune response to the LSN antigen in mice that were inoculated with the LSN replicon. Maintained animal husbandry. (Exhibit O, ¶14).

February 11, 1995 (Saturday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on February 14, 1995.

February 12, 1995 (Sunday): Maintain ongoing animal study of Lassa replicon with animal husbandry to the next step on February 14, 1995.

February 13, 1995 (Monday): Maintain ongoing animal study of Lassa replicon with animal husbandry to the next step on February 14, 1995.

February 14, 1995 (Tuesday): Performed Plaque Reduction Neutralization test of serum samples against V3000. Protocol followed was NEUTASSY.PRO. (Exhibit O, ¶15).

February 15, 1995 (Wednesday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on February 20, 1995.

February 16, 1995 (Thursday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on February 20, 1995.

February 17, 1995 (Friday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on February 20, 1995.

February 18, 1995 (Saturday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on February 20, 1995.

February 19, 1995 (Sunday): Maintain ongoing animal study of Lassa replicon with animal husbandry to the next step on February 20, 1995.

February 20, 1995 (Monday): Conducted the 60-day post-inoculation bleed. Samples were stored at -20°C in freezer in Room 304. (Exhibit O, ¶16).

February 21, 1995 (Tuesday): Maintained ongoing animal study of Lassa replicon with animal

husbandry to the next step on March 21, 1995.

February 22, 1995 (Wednesday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on March 21, 1995.

February 23, 1995 (Thursday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on March 21, 1995.

February 24, 1995 (Friday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on March 21, 1995.

February 25, 1995 (Saturday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on March 21, 1995.

February 26, 1995 (Sunday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on March 21, 1995.

February 27, 1995 (Monday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on March 21, 1995.

February 28, 1995 (Tuesday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on March 21, 1995.

March 1, 1995 (Wednesday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on March 21, 1995.

March 2, 1995 (Thursday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on March 21, 1995.

March 3, 1995 (Friday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on March 21, 1995.

March 4, 1995 (Saturday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on March 21, 1995.

March 5, 1995 (Sunday): Maintained ongoing animal study of Lassa replicon with animal

husbandry to the next step on March 21, 1995.

March 6, 1995 (Monday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on March 21, 1995.

March 7, 1995 (Tuesday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on March 21, 1995.

March 8, 1995 (Wednesday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on March 21, 1995.

March 9, 1995 (Thursday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on March 21, 1995.

March 10, 1995 (Friday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on March 21, 1995.

March 11, 1995 (Saturday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on March 21, 1995.

March 12, 1995 (Sunday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on March 21, 1995.

March 13, 1995 (Monday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on March 21, 1995.

March 14, 1995 (Tuesday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on March 21, 1995.

March 15, 1995 (Wednesday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on March 21, 1995.

March 16, 1996 (Thursday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on March 21, 1995.

March 17, 1995 (Friday): Maintained ongoing animal study of Lassa replicon with animal

husbandry to the next step on March 21, 1995.

March 18, 1995 (Saturday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on March 21, 1995.

March 19, 1995 (Sunday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on March 21, 1995.

March 20, 1995 (Monday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on March 21, 1995.

March 21, 1995 (Tuesday): Tested the Lassa ELISA antigen for appropriate dilution. The antigen was prepared in a standard carbonate/bicarbonate buffer. Two-fold dilutions were done across a 96 well ELISA plate in all rows starting at 1:100. Two plates were prepared. Maintained animal husbandry. (Exhibit O, ¶17).

March 22, 1995 (Wednesday): Tested the Lassa ELISA antigen for appropriate dilution. The antibodies were obtained from either normal mouse serum or undiluted immunized serum (day 37 from mouse #4). Two-fold dilutions were prepared on 96 well plates. Maintained animal husbandry. (Exhibit O, ¶18).

March 23, 1995 (Thursday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on March 26, 1995.

March 24, 1995 (Friday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on March 26, 1995.

March 25, 1995 (Saturday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on March 26, 1995.

March 26, 1995 (Sunday): Performed Lassa ELISA with the mouse serum from the above experiment. Protocol followed was ELISA2.PRO. Antigen plates were prepared. Dilution was 1:6000 in carbonate/bicarbonate buffer. Maintained animal husbandry. (Exhibit O, ¶19).

March 27, 1995 (Monday): Completed Lassa ELISA with the mouse serum from the above experiment completed as per ELISA2.PRO protocol. (Exhibit O, ¶20).

March 28, 1995 (Tuesday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on April 24, 1995.

March 29, 1995 (Wednesday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on April 24, 1995.

March 30, 1995 (Thursday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on April 24, 1995.

March 31, 1995 (Friday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on April 24, 1995.

April 1, 1995 (Saturday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on April 24, 1995.

April 2, 1995 (Sunday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on April 24, 1995.

April 3, 1995 (Monday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on April 24, 1995.

April 4, 1995 (Tuesday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on April 24, 1995.

April 5, 1995 (Wednesday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on April 24, 1995.

April 6, 1995 (Thursday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on April 24, 1995.

April 7, 1995 (Friday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on April 24, 1995.

April 8, 1995 (Saturday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on April 24, 1995.

April 9, 1995 (Sunday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on April 24, 1995.

April 10, 1995 (Monday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on April 24, 1995.

April 11, 1995 (Tuesday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on April 24, 1995.

April 12, 1995 (Wednesday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on April 24, 1995.

April 13, 1995 (Thursday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on April 24, 1995.

April 14, 1995 (Friday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on April 24, 1995.

April 15, 1995 (Saturday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on April 24, 1995.

April 16, 1995 (Sunday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on April 24, 1995.

April 17, 1995 (Monday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on April 24, 1995.

April 18, 1995 (Tuesday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on April 24, 1995.

April 19, 1995 (Wednesday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on April 24, 1995.

April 20, 1995 (Thursday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on April 24, 1995.

April 21, 1995 (Friday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on April 24, 1995.

April 22, 1995 (Saturday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on April 24, 1995.

April 23, 1995 (Sunday): Maintained ongoing animal study of Lassa replicon with animal husbandry to the next step on April 24, 1995.

April 24, 1995 (Monday): I performed Plaque Reduction Neutralization test of serum samples against V3000 using the protocol NEUTASSY.PRO. (Exhibit O, ¶21).

April 25, 1995 (Tuesday) **through May 23, 1995** (Tuesday): Maintained ongoing animal study of Lassa replicon with animal husbandry for next step on July 11, 1995.

13. The work conducted from before November 30, 1994 continuously through May 23, 1995 demonstrates unequivocally in my opinion, that RNA Replicon Systems developed before and after November 30, 1994 involving particles carrying RNA encoding an immunogen, in fact generate the immune response my earlier work had demonstrated was a reasonable expectation.

14. The immune response data gained from the entirety of the LSN replicon animal trial initiated on December 22, 1994 and continuing uninterrupted until the conclusion of the trial well after May 23, 1995 could not have been obtained more quickly than as outlined above or without daily observation and care of the involved animals. This is generally true for any animal trial designed to evaluate the immune response of potential vaccines. In the case of the LSN replicon trial it was necessary to observe the animals daily for any sign of illness or distress, and to obtain serum titers at time points distant from the initial immunization and subsequent boost, at least in part to observe any change in the immunoglobulin titer or specificity for LSN that could

potentially indicate poor efficacy or a change in the VEE immunoglobulin titer that could be suggestive of the presence of replication competent VEE virus that is capable of initiating a productive infection. The latter result can be masked by the initial immune response to the VEE particle proteins, and therefore, must be followed over time. In addition, monitoring the animals will indicate if there is a drop in the immunogen titer, and the animals can be boosted at late time points and titers monitored subsequently. This is one standard method by which immunization protocols are defined, so that titers never drop below protective levels.

I, George V. Ludwig, Ph.D. declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that any such willful false statements may jeopardize the validity of U.S. Patent Application No. 10/683,781 or any patent issued thereon.

19 August 2004
Date

George V. Ludwig, Ph.D.